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USPT,JPAB,EPAB,DWPI	l2 and vector	3200	L3
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USPT,JPAB,EPAB,DWPI	Homologous recombination	5846	L1

J Virol 1997 Oct;71(10):7820-6

Development of high-titer retroviral producer cell lines by using Cre-mediated recombination.

Vanin EF, Cerruti L, Tran N, Grosveld G, Cunningham JM, Jane SM

Rotary Bone Marrow Research Laboratory, Royal Melbourne Hospital Research Foundation, Parkville, Victoria, Australia.

Retroviral gene transfer is widely used in experimental and human gene therapy applications. We have devised a novel method of generating high-titer retroviral producer cell lines based on the P1 bacteriophage recombinase system Cre-loxP. Incorporation of loxP sites flanking a Neo(r)-SVTK cassette in the proviral DNA allows excision of these selectable markers through expression of Cre recombinase after production of a high-titer producer cell line. The resultant producer line contains a single loxP site flanked by the viral long terminal repeats. Retransfection of this line with the Cre expression vector and a plasmid containing a gene of interest flanked by loxP sites allows insertional recombination of the gene into the favorable preexisting site in the genome and the generation of a new line with a titer equivalent to that of the parental producer cell line. The efficiency of the process is sufficient to allow the generation of multiple new producer lines without the addition of antibiotic resistance genes. We have successfully generated retroviral vectors carrying different genes by using this approach and discuss the potential applications of this method in gene therapy.

PMID: 9311869, UI: 97456555

Proc Natl Acad Sci U S A 1996 Aug 20;93(17):8971-6

Reversible immortalization of mammalian cells mediated by retroviral transfer and site-specific recombination.

Westerman KA, Leboulch P

Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Cambridge 02139, USA.

A procedure of reversible immortalization of primary cells was devised by retrovirus-mediated transfer of an oncogene that could be subsequently excised by site-specific recombination. This study focused on the early stages of immortalization: global induction of proliferation and life span extension of cell populations. Comparative analysis of Cre/LoxP and FLP/FRT recombination in this system indicated that only Cre/LoxP operates efficiently in primary cells. Pure populations of cells in which the oncogene is permanently excised were obtained, following differential selection of the cells. Cells reverted to their preimmortalized state, as indicated by changes in growth characteristics and p53 levels, and their fate conformed to the telomere hypothesis of replicative cell senescence. By permitting temporary and controlled expansion of primary cell populations without retaining the transferred oncogene, this strategy may facilitate gene therapy manipulations of cells unresponsive to exogenous growth factors and make practical gene targeting by homologous recombination in somatic cells. The combination of retroviral transfer and site-specific recombination should also extend gene expression studies to situations previously inaccessible to experimentation.

PMID: 8799138, UI: 96392350

1: Bioessays 1998 Jul;20(7):532-5 Related Articles, Links

InterScience

Conspiracy of silence among repeated transgenes.

Henikoff S.

Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024, USA. steven@muller.fhcrc.org

Transgenic experiments in vertebrates often involve the insertion of tandem multiple-copy arrays at single sites. For many transgenes, expression is unpredictable from site to site, a phenomenon usually attributed to a repressive environment caused by nearby sequences. However, an alternative explanation comes from evidence that transgene repeat arrays in flies condense into heterochromatin, suggesting that low levels of expression in vertebrate transgene arrays might result from interactions between repeats within the array. A recent experiment using transgenic mouse lines demonstrates that reduction in copy number of silenced transgenes within an array leads to a striking increase in expression, demonstrating that silencing is intrinsic to the array, and is not attributable to position effects of nearby sequences. This work calls into question functions that have been attributed to vertebrate locus control regions and boundaries, and draws attention to the notion that repeat-induced gene silencing is a system for protection of eukaryotic genomes against threatening sequence elements.

Publication Types:
- Review
- Review, Tutorial

PMID: 9723001 [PubMed - indexed for MEDLINE]

2: Nat Genet 1998 Jan;18(1):56-9 Related Articles, Links

Comment in:
- Nat Genet. 1998 Jan;18(1):5-6.

1: Proc Natl Acad Sci U S A 1996 Aug 20;93(17):9067-72

Comment in:

- Proc Natl Acad Sci U S A. 1996 Aug 20;93(17):8824-8

Single-copy transgenic mice with chosen-site integration.

Bronson SK, Plaehn EG, Kluckman KD, Hagaman JR, Maeda N, Smithies O.

Department of Pathology, University of North Carolina, Chapel Hill 27599-7525, USA.

We describe a general way of introducing transgenes into the mouse germ line for comparing different sequences without the complications of variation in copy number and insertion site. The method uses homologous recombination in embryonic stem (ES) cells to generate mice having a single copy of a transgene integrated into a chosen location in the genome. To test the method, a single copy murine bcl-2 cDNA driven by either a chicken beta-actin promoter or a human beta-actin promoter has been inserted immediately 5' to the X-linked hypoxanthine phosphoribosyltransferase locus by a directly selectable homologous recombination event. The level of expression of the targeted bcl-2 transgene in ES cells is identical in independently isolated homologous recombinants having the same promoter yet varies between the different promoters. In contrast, the expression of bcl-2 transgenes having the same (chicken beta-actin) promoter varies drastically when they are independently integrated at random insertion sites. Both promoters direct broad expression of the single-copy transgene in mice derived from the respective targeted ES cells. In vitro and in vivo, the human beta-actin promoter consistently directed a higher level of transgene expression than the chicken beta-actin promoter.

PMID: 8799155 [PubMed - indexed for MEDLINE]

- Review, Tutorial

PMID: 10999408 [PubMed - indexed for MEDLINE]

☐7: Plant Mol Biol 2001 Jul;46(4):433-45 Related Articles, NEW! Links

Transgene silencing of invertedly repeated transgenes is released upon deletion of one of the transgenes involved.

De Buck S, Van Montagu M, Depicker A.

Departement Plantengenetica, Vlaams Interuniversitair Instituut voor Biotechnologie, Universiteit Gent, Belgium.

To analyse experimentally the correlation between transgene silencing and the presence of an inverted repeat in transgenic Arabidopsis thaliana plants, expression of the beta-glucuronidase (gus) gene was studied when present as a convergently transcribed inverted repeat or as a single copy in otherwise isogenic lines. In transformants containing two invertedly repeated gus genes separated by a 732 bp palindromic sequence, gus expression was low, as exemplified by the expression levels in the parental line KH15. The parental KH15 locus could induce efficiently in trans silencing of gus copies at allelic and non-allelic positions. In transformants containing two invertedly repeated gus genes separated by a 826 bp non-repetitive spacer region, gus expression was high or intermediate, especially in hemizygous state and at late developmental stages, as demonstrated in detail for line KHsb67. Removal of one of the gus copies by Cre recombinase resulted in all cases in constitutively high gus expression in hemizygous as well as in homozygous state. The derived deletion lines could no longer induce in trans silencing of homologous gus copies. The results show that convergent transcription of transgenes in an inverted repeat is an important parameter to trigger their silencing and that co-transformation of two T-DNAs with identical transgenes can be used to obtain inverted repeats and targeted co-suppression of the homologous endogenes. Moreover, the data suggest that the spacer region in between the inverted genes plays a role in the efficiency of initiating and maintaining silencing.

PMID: 11485200 [PubMed - indexed for MEDLINE]

Dorer DR, Henikoff S.

Howard Hughes Medical Institute, Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104.

Closely linked repeats of a Drosophila P transposon carrying a white transgene were found to cause white variegation. Arrays of three or more transgenes produced phenotypes similar to classical heterochromatin-induced position-effect variegation (PEV), and these phenotypes were modified by known modifiers of PEV. This effect on the repeated transgenes was much stronger for a site near centric heterochromatin than it was for a medial site, and it strengthened with increasing copy number. Differences between variegated phenotypes could be accounted for if different topological structures were generated by pairing between closely linked repeat sequences. We propose that pairing of repeats underlies heterochromatin formation and is responsible for diverse gene silencing phenomena in animals and plants.

PMID: 8020105 [PubMed - indexed for MEDLINE]

☐ **6:** Plant Mol Biol 2000 Jun;43(2-3):243-60 Related Articles, NEW Links

Role of inverted DNA repeats in transcriptional and post-transcriptional gene silencing.

Muskens MW, Vissers AP, Mol JN, Kooter JM.

Department of Developmental Genetics, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, Netherlands.

Transgenes and endogenous genes are sensitive to silencing, in particular when the genes are tandemly repeated. Their expression can be transcriptionally or post-transcriptionally repressed, or both. It is remarkable that very often, two or more genes or parts of the genes are arranged as inverted repeats (IR). Many of such IRs are dominant silencing loci. They can repress the expression of homologous genes elsewhere in the genome in trans which is usually associated with an increase in the level of DNA methylation. Trans-silencing has been explained by DNA-DNA pairing between a repetitive silencing locus and a homologous target locus. However, there is accumulating evidence that the trans effect might be mediated by dsRNA transcribed from the IR (trans)genes. Besides dsRNA-directed DNA methylation, dsRNA in plants as well as in other systems also induces the degradation of homologous RNAs and silence genes post-transcriptionally. These findings indicate that several features associated with gene silencing can be attributed to the activities of dsRNA, which would explain why inverted transgene repeats are such efficient silencing loci.

Publication Types:
- Review

enhanced variegation, and enhancement was reverted by recombination of the array onto a normal sequence chromosome. Rearrangements that lack the array enhanced variegation of white on a homologue bearing the array. Therefore, silencing of white genes within a repeat array depends on its distance from heterochromatin of the same chromosome or of its paired homologue. In addition, white transgene arrays cause variegation of a nearby gene in cis, a hallmark of classical position-effect variegation. Such spreading of heterochromatic silencing correlates with array size. Finally, white transgene arrays cause pairing-dependent silencing of a non-variegating white insertion at the homologous position.

PMID: 9383061 [PubMed - indexed for MEDLINE]

☐4: Bioessays 1996 Nov;18(11):919-23 Related Articles, NEW Links

The vagaries of variegating transgenes.

Martin DI, Whitelaw E.

Fred Hutchinson Cancer Research Center, Seattle, WA 98104, USA. dmartin@fred.fhcrc.org

Expression of transgenes in mice, when examined with assays that can distinguish individual cells, is often found to be heterocellular, or variegated. Line-to-line variations in expression of a transgene may be due largely to differences in the proportion of cells in which it is expressed. Variegated silencing by centromeric heterochromatin is well described, but other factors may also affect transgene silencing in mice. Tandem arrays of transgenes themselves form heterochromatin, and some cell lineages may tend to silence transgenes because of extensive facultative heterochromatin in their nuclei. The cis-acting transcriptional control elements within a transgene inhibit silencing, and strain-specific differences in chromatin proteins may strongly influence the extent of variegation. The accessibility of multiple differentiated cell lineages in mice suggests that they may provide a tool for dissecting the role of chromatin-mediated silencing in cell differentiation and tissue-specific gene expression.

Publication Types:
- Review
- Review, Tutorial

PMID: 8939070 [PubMed - indexed for MEDLINE]

☐5: Cell 1994 Jul 1;77(7):993-1002 Related Articles, NEW Links

FULL TEXT @ CELL PRESS

Expansions of transgene repeats cause heterochromatin formation and gene silencing in Drosophila.

Repeat-induced gene silencing in mammals.

Garrick D, Fiering S, Martin DI, Whitelaw E.

Department of Biochemistry, University of Sydney, New South Wales, Australia.

In both plants and Drosophila melanogaster, expression from a transgenic locus may be silenced when repeated transgene copies are arranged as a concatameric array. This repeat-induced gene silencing is frequently manifested as a decrease in the proportion of cells that express the transgene, resulting in a variegated pattern of expression. There is also some indication that, in transgenic mammals, the number of transgene copies within an array can exert a repressive influence on expression, with several mouse studies reporting a decrease in the level of expression per copy as copy number increases. However, because these studies compare different sites of transgene integration as well as arrays with different numbers of copies, the expression levels observed may be subject to varying position effects as well as the influence of the multicopy array. Here we describe use of the lox/Cre system of site-specific recombination to generate transgenic mouse lines in which different numbers of a transgene are present at the same chromosomal location, thereby eliminating the contribution of position effects and allowing analysis of the effect of copy number alone on transgene silencing. Reduction in copy number results in a marked increase in expression of the transgene and is accompanied by decreased chromatin compaction and decreased methylation at the transgene locus. These findings establish that the presence of multiple homologous copies of a transgene within a concatameric array can have a repressive effect upon gene expression in mammalian systems.

PMID: 9425901 [PubMed - indexed for MEDLINE]

☐3: Genetics 1997 Nov;147(3):1181-90 Related Articles, NEW Links

Transgene repeat arrays interact with distant heterochromatin and cause silencing in cis and trans.

Dorer DR, Henikoff S.

Howard Hughes Medical Institute, Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109-1024, USA.
dorerd01@ccvax.mmc.edu

Tandem repeats of Drosophila transgenes can cause heterochromatic variegation for transgene expression in a copy-number and orientation-dependent manner. Here, we demonstrate different ways in which these transgene repeat arrays interact with other sequences at a distance, displaying properties identical to those of a naturally occurring block of interstitial heterochromatin. Arrays consisting of tandemly repeated white transgenes are strongly affected by proximity to constitutive heterochromatin. Moving an array closer to heterochromatin